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Changes in the Fungal Autoflora of Apollo Astronauts

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Specimens were repeatedly obtained for mycological examination from the skin, throat, urine, and feces of the six astronauts who conducted the Apollo 14 and Apollo 15 lunar exploration missions. Analysis of preflight data demonstrates that the process of severely restricting opportunities from colonization for 3 weeks before flight resulted in a 50% reduction in the number of isolated species. Postflight data indicate that exposure to the space flight environment for up to 2 weeks resulted in an even greater reduction with a relative increase in the potential pathogen *Candida albicans*. No incidences of microbial shock were observed when crewmembers were quarantined for 16 days after completion of the space flight. Intercrew transfer of particular species could not be demonstrated because most species were not consistently recovered.

Although the indigenous fungal flora of healthy male adults has not been extensively studied, an understanding of the qualitative and quantitative fungal burden of astronauts is of paramount importance for developing effective preventive measures against the occurrence of infectious disease during space flight missions (18, 20, 34, 35, 37). Because disease events may be either from exogenous or endogenous sources, the fungal autoflora present in specific body areas of each of the crewmembers was carefully monitored before and after the Apollo 14 and 15 space flights. The resulting data were evaluated to determine any observable changes that the conditions of space flight may have imposed upon the recoverable autoflora of the subject astronauts.

In an attempt to protect astronauts from inflight manifestations of disease originating from infections of exogenous orgin which were initiated before flight, each subject was maintained in a controlled environment the last 21 days before launch. This condition was imposed to allow the autoflora to equilibrate at a level consistent with confinement and to allow contracted infectious agents to demonstrate themselves before flight. The actual influence of this isolation procedure on the fungal autoflora of the crewmembers is described and evaluated.

In addition to the more obvious exogenous diseases which may afflict crewmembers, the importance of endogenous infections has been identified by several authors (7, 8, 14, 15, 20, 21, 26, 41, 43; L. S. Gall and P. E. Riely, Bacteriol. Proc., p. 7-8, 1966). Concern for infections involving endogenous microorganisms is based upon the recognition that the unusual environment offered by the spaceship may so influence the indigenous microflora that pathologic activity may be demonstrated by certain microbes which did not demonstrate such activity during the preflight observation period.

An associated concern is for the possibility of an unusually severe response of the returning astronauts when they are again subjected to exogenous sources of infection after the flight (8, 26, 34, 37, 41). This hypothetical phenomenon, occasionally referred to as "microbial shock" (2, 25), could result in the precipitous and harmful action of the newly encountered microorganisms and/or their products on the returning astronauts (26). Accordingly, careful attention was given to the degree of autoflora reduction during spaceflight and the subsequent reaction upon reintroduction into the earth environment. Data obtained from analysis of Apollo 14 and 15 crew specimens were collected and analyzed in such a way that the areas of concern outlined above could be evaluated for 2-week space flight missions.

MATERIALS AND METHODS

Samples collected. Twelve samples were collected from each of the six subject astronauts during each of

the collection periods as previously outlined (38). Eight of these were swabs of body surface areas which included: (i) scalp—a 13-cm² area below the hairline at the base of the neck, (ii) ears—right and left external auditory canals, (iii) axillae—a 6.5-cm² area below the hairline on each side, (iv) hands—a 6.5-cm² area in the center of both palms (v) navel—the internal area of the umbilicus with a surrounding area of 13 cm², (vi) groin—a 5-cm strip from the rear to the front on the right and left inguinal area, (vii) toes—area between the large and second toe of each foot, and (viii) nares—entire surface of the left and right outermost nasal chamber. Calcium alginate swabs (Wilson Diagnostics, Inc., Glenwood, Ill.), wetted in 0.3 mM phosphate buffer, were used to sample each of these body surface areas. This swab technique was chosen as the only collection method compatible with the flight program. Although it is recognized that subsurface fungi may be overlooked by this method, procedures for sampling subsurface areas were not acceptable. Therefore, data must be interpreted in terms of surface inhabitants.

The other four samples, collected from each astronaut, include a throat swab, a throat gargle-mouth wash sample, a urine sample, and a fecal sample. Dry calcium alginate swabs were used to sample the surfaces of tonsils and posterior pharyngeal vault prior to the collection of the gargle specimen. For this latter sample, the subjects gargled with a 0.3 mM phosphate buffer solution, followed by a repeated rinse of the teeth with the same solution. Midstream urine samples were collected into a sterile container with the first void of the morning. Fecal samples were collected into a sterile cardboard container at the convenience of the subject.

Sample collection intervals. Five different sets of specimens were collected from the Apollo 14 crewmembers. The first was taken 30 days before flight (F − 30), and the second was taken 2 weeks later (F − 15) after the astronauts had been in a preflight controlled environment for 6 days. Another set of samples was secured the morning of launch (F − 0), and the fouth was taken when the crewmembers reached the recovery ship after splashdown (R + 0). The last set of samples was taken the day the crewmembers were released from their postflight isolation, 16 days after recovery (R + 16).

Only four sets of specimens were collected from the Apollo 15 crewmembers, for there was no postflight quarantine and the R + 16 day set was deleted. In addition, an F − 5 day set was added to the Apollo 15 protocol in place of the F − 15 day sample set. All of the preflight samples and the R + 16 sample set were collected when the astronauts arose in the morning and before any soap, tooth paste, or deodorant had been used. The immediate postflight samples (R + 0) were collected within 30 min of the time the crewmembers were brought aboard the recovery ship.

Specimen analysis. Swabs were placed in Trypticase soy broth (TSB, Baltimore Biological Laboratories) and maintained on wet ice for 10 h before processing. This delay was imposed in an attempt to equalize variations caused by shipment from remote collection sites. Portions of each sample were diluted in TSB as appropriate and subsequently streaked for isolation on the following agar media: (i) corn meal-malt-yeast extract agar plus antibiotics (20,000 U of penicillin G per liter plus 40,000 μg of streptomycin per liter), (ii) Sabouraud dextrose agar (Difco) plus antibiotics (20,000 U of penicillin G per liter plus 40,000 μg of streptomycin per liter), and (iii) Czapek-Dox agar (Difco). After incubation at 25 C for 120 h. representatives of each morphologically distinct colony type were picked to identification media.

The remainder of each original sample was centrifuged at 12,000 × *g* (5,000 rpm) for 15 min. The supernatant fluid was discarded, and the resulting sediment was used to streak culture plates containing isolation media plus antibiotics as above. All resulting colonies which were morphologically different from those isolated from the original dilution plates, if any, were picked from the agar surface for identification. All isolates were identified to genus, and to species where possible, by employing current keys. Species occurring in unusually high numbers were noted.

Environmental parameters. The crewmembers were subjected to several different types of environments throughout the monitoring period as previously described (38). Samples were collected during each of these periods so that resulting changes in the mycotic autoflora could be detected and related to the known external conditions.

Prior to launch the crewmembers were maintained in two quite different environments commencing with an extended period characterized by an absence of any environmental controls. During the last 3 weeks before launch, the crewmembers were restricted to several special areas at the Kennedy Space Center which had been modified so that strict control of airborne microbial contaminants could be achieved and monitored. Contact with contaminating fomites and food were guarded against, and only a limited number of carefully selected people were allowed access to the astronauts (42).

During the space flight, the astronauts were restricted to the environment of their spacecrafts (command module and lunar module) and extra vehicular activity clothing. Immediately upon recovery the crewmembers entered the sick bay of the prime recovery ship where specimens were obtained. These specimens were returned to the Johnson Space Center where their microbial load was studied. All Apollo 14 postflight sample analyses were conducted within class III biological glove boxes in the lunar receiving laboratory at the Johnson Space Center to avoid possible introduction of lunar contaminants into the terrestrial biosphere.

Upon termination of their 10-day space flight, and while aboard the prime recovery ship, the Apollo 14 astronauts entered the isolated environment of the mobile quarantine facility where they remained throughout the 58-h trip to the Johnson Space Center. They were then transferred to the crew reception area of the Lunar Receiving Laboratory for the remainder of the 16 days of postflight quarantine. During this time contact with outside sources of

contamination was strictly prohibited, although there was frequent contact with the 18 attendant personnel who were quarantined with the astronauts in the crew reception area.

RESULTS

A summary of the genera of yeasts and filamentous fungi isolated from crew specimens throughout the Apollo 14 and 15 missions is presented in Table 1. Thirty-six specimens (12 specimens per astronaut × 3 astronauts) were collected at each sample period. Because there were nine sample periods for the two missions, there was a total of 324 specimens examined mycologically. In all, 112 species, belonging to 57 genera, were identified from these specimens. Fifty-three percent (27) of these genera were recovered during no more than one period per mission and are marked as rarely isolated genera in Table 1. Of the remaining 27 genera, 13 were isolated frequently (during four or more sample periods). However, in the case of the genera *Aspergillus* and *Penicillium*, there were many different representatives within the genus, so that most species of these genera were actually rarely isolated. For example none of the 11 species of *Aspergillus* was isolated during more than one sample period, indicating the true transient relationship of these species. The number of isolates recovered during each sample period is indicated both as total reported isolates and total number of genera.

For the purposes of this paper, only those species which were isolated frequently (recovered at least once during four or more sample periods) are considered in detail. A complete listing of these 10 species is presented in Table 2. These include one species each from the genera *Aureobasidium*, *Pityrosporum*, and *Scolescobasidium*, with all others belonging to the genera *Candida* and *Cladosporium*. *Candida albicans* was the most ubiquitous microorganism, being recovered during each of the nine sample periods a total of 42 times. Other species of *Candida* were also frequently isolated, with the four species listed in Table 2 accounting for 60% of all the isolates outlined on this table. Similarly, three species of *Cladosporium* account for 26% of all isolates from the frequently occurring microorganisms presented in Table 2. *Scolescobasidium verruculosm* was the least frequently isolated species of this group, being recovered from the Apollo 14 crew only. A tabulation of the mycological recovery of all crewmembers for both missions is presented in Table 3.

Because the frequently isolated species recovered throughout the Apollo 14 and 15 missions are restricted to five genera, only these genera were evaluated for specific variations throughout the two missions. All of the members of these five genera, isolated during the Apollo 14 and 15 missions, are presented in Tables 4 and 5 respectively. These data indicate from which site, crewmember, and sample period each species was recovered.

DISCUSSION

Because little work has been done on the fungi commonly recovered from healthy adult males, we have considered it important to evaluate the fungal load of Apollo crewmembers in an attempt to better understand the importance of this parameter in space flight. Accordingly we repeatedly secured samples from 12 representative body areas of each of six Apollo astronauts. Each of the filamentous fungi and yeasts recovered from these samples were identified to species where possible so that subsequent evaluations could be made at this level.

All of the identified isolates, grouped according to genus, are presented in Table 1. When only the genera are considered, these data reveal that whereas a large number of different types of fungi (representatives of 57 genera) were isolated from these healthy male adults, the majority (53%) were isolated no more than once during a particular Apollo mission. These numbers compare well with the 53 "fungal or yeast varieties" isolated from aquanauts prior to the Tektite I dive (24). Even though the low recovery rate expressed by these particular genera indicates that they are most properly considered as environmental contaminants, their evaluation was important to our study since many fungi which are pathogenic to man are soil saprophytes (1, 17) and could be among the environmental contaminants temporarily residing on the body surface before flight (14, 20, 34). Accordingly, enumeration of all isolates was performed in an attempt to evaluate the effect of the space flight and quarantine environments on both the indigenous and the transient fungal flora.

Each recovered isolate was identified to species where possible so that accurate interpretations of fungal population dynamics could be made. A common practice, when evaluating fungal loads of human subjects confined in spacecraft simulations, has been to report recovered genera only. By using this approach, the genera *Aspergillus* and *Penicillium* have been reported as major contributors to the autoflora of man (24, 36).

However, examination of our data demonstrates that although these genera are often recovered, a particular species is not commonly

TABLE 1. *Number of fungal isolates recovered from Apollo 14 and 15 crews during each sample period*

Genus	No. of species	Apollo 14 sample periods					Apollo 15 sample periods				No. of times isolated	Genus[a] rarely isolated	Genus[b] frequently isolated
		F − 30	F − 15	F − 0	R + 0	R + 16	F − 30	F − 5	F − 0	R + 0			
Acromonium	1	—	1[c]	—	—	—	—	—	—	—	1	+	
Alternaria	2	1	—	—	—	1	2	—	2	—	6		+
Aphanoascus	3	—	—	—	—	—	4	—	—	—	4	+	
Arthrinium	1	1	—	—	—	—	—	—	—	—	1	+	
Aspergillus	11	—	2	5	—	3	2	2	1	—	15		+
Aureobasidium	1	—	—	1	—	—	1	1	3	—	6		+
Bipolaris	1	—	1	—	—	—	1	—	—	—	2	+	
Candida	8	17	8	7	4	4	14	15	12	6	87		+
Cephaloascus	1	—	—	—	—	1	—	—	—	—	1	+	
Cephalosporium	1	—	—	1	—	—	—	—	—	—	1	+	
Chaetomium	1	—	—	—	—	—	—	—	—	1	1	+	
Chrysosporium	1	10	—	1	—	—	—	—	—	—	11		
Cladorrhinum	1	1	2	—	—	—	—	—	—	—	3		
Cladosporium	7	7	9	8	—	19	3	4	—	—	50		+
Coniella	1	1	—	—	—	—	—	—	—	—	1	+	
Coniothyrium	1	2	—	—	—	1	—	—	—	—	3		
Cryptococcus	1	6	—	—	—	—	—	1	—	—	7	+	
Curvularia	1	—	—	—	—	—	—	—	1	—	1	+	
Diplococcium	1	—	1	1	—	—	—	—	—	—	2		
Emericellopsis	1	1	—	—	—	—	—	—	—	—	1	+	
Epicoccum	1	1	—	—	—	—	1	—	—	—	2	+	
Fusarium	1	—	1	—	—	1	—	—	—	—	2		
Fusidium	1	1	—	—	—	—	4	—	—	—	5	+	
Geotricium	2	3	2	4	1	2	5	—	1	—	18		+
Haplobasidion	2	—	1	—	—	—	—	1	1	—	3		
Hyalodendron	1	—	—	—	—	—	1	—	—	—	1	+	
Kabatiella	1	—	—	—	—	1	—	—	—	—	1	+	
Microthecium	1	1	—	—	—	—	—	—	—	—	1	+	
Mucor	1	1	—	—	—	—	—	—	—	—	1	+	
Nigrosporia	3	1	—	—	—	—	3	2	1	—	7		+
Oidiodendron	1	1	—	—	—	4	—	—	—	—	5		
Paecilomyces	6	3	—	—	—	1	1	1	—	—	6		+
Penicillium	10	9	4	4	2	4	7	3	—	2	35		+
Periconia	4	1	4	—	—	1	—	—	—	—	6		
Philophora	1	—	1	—	—	—	—	—	—	—	1	+	
Phoma	1	2	—	—	—	—	—	1	1	—	4		
Pichia	1	—	—	—	—	—	1	2	—	—	3		
Pithomyces	2	1	—	—	—	—	1	—	—	—	2	+	
Pityrosporum	1	5	1	1	—	—	—	—	1	—	8		+
Rhinocladiella	1	—	—	—	—	—	—	—	—	1	1	+	
Rhodotorula	4	2	—	—	—	—	1	1	1	1	6		+
Saccharomyces	2	—	—	1	—	—	—	1	1	—	3		
Scolescobasidium	1	1	1	1	—	1	—	—	—	—	4		+
Septonema	1	1	—	—	—	—	—	—	—	—	1	+	
Sporothrix	1	—	1	2	—	—	—	—	—	—	3		
Staphylotrichum	1	1	—	—	—	—	—	—	—	—	1	+	
Sterigmatomyces	1	1	—	—	—	—	—	—	—	—	1	+	
Stilbum	1	—	1	—	—	—	—	—	—	—	1	+	
Syncephalastrum	1	—	2	—	—	—	—	—	—	—	2	+	
Thysanophora	1	—	1	—	—	—	—	—	—	—	1	+	
Tilletiopsis	1	—	—	—	—	1	2	—	1	—	4		
Torula	2	1	1	—	—	—	1	—	—	—	3		
Torulomyces	1	1	—	—	—	—	—	—	—	—	1	+	
Torulopsis	3	—	1	—	—	—	1	1	2	—	5		+
Trichoderma	1	—	—	—	—	—	—	4	—	—	4	+	
Wallemia	1	—	2	—	—	—	—	—	—	—	2	+	
Zygosporium	1	—	—	—	—	—	1	—	—	—	1	+	
Total no. of isolates		85	48	37	7	45	57	40	29	11			
Total no. of genera		30	22	13	3	15	21	15	14	5		30	13

[a] Isolated no more than once per mission.
[b] Isolated four or more times.
[c] Number of times isolated per sample period.

isolated more than once. The same pattern was observed for members of the genera *Nigrospora* and *Paecilomyces*. These data indicate that members of these four genera are truly transient, and recovery incidences may be expected to fluctuate in a manner similar to the other groups which are not part of the permanent autoflora.

For the purposes of this study, a species was considered frequently isolated if it was recovered at least once during four or more sample periods. Of the 10 qualifying species outlined in Table 2 the species *C. albicans* was isolated during each of the nine sample collection periods a total of 42 times and is clearly a constant contributor to the total autoflora. Although included in this table, the species *Cladosporium elatum* and *S. verruculosum* appear to be of minor importance because they were isolated from only one crew. Of the fungi which are commonly recovered from healthy adults, only the potential pathogen, *C. albicans* has definitely been established as an indigenous species (32), although other species of *Candida*, as well as *Pityrosporum ovale*, have been isolated from human sources more frequently than from nonhuman sources, and thereby may yet prove to be part of the indigenous flora of man (10, 11, 12, 29).

The data presented in this paper support this view. In addition, the isolation frequency of *Candida parapsilosis* and *Candida tropicalis* indicate that these species should be thoroughly studied pending consideration as members of the indigenous fungal autoflora of healthy male adults. *Cladosporium herbarum* was likewise

TABLE 2. *Fungal species isolated during at least four of the nine Apollo 14 and 15 sample periods*

Genus and species	Sample periods									Total times recovered (out of 9)	Total no. of isolates
	Apollo 14					Apollo 15					
	F − 30	F − 15	F − 0	R + 0	R + 16	F − 30	F − 5	F − 0	R + 0		
Aureobasidium pullulans	0[a]	0	1	0	0	1	1	3	0	4	6
Candida albicans	2	6	6	3	4	5	6	5	5	9	42
Candida parapsilosis	2	1	0	0	0	7	4	3	0	5	17
Candida tropicalis	8	0	0	0	0	1	2	1	1	5	13
Candida krusei	0	1	0	0	0	1	2	2	0	4	6
Cladosporium cladosporioides	2	0	2	0	3	1	1	0	0	5	9
Cladosporium elatum	1	1	1	0	3	0	0	0	0	4	6
Cladosporium herbarum	0	5	2	0	6	2	3	0	0	5	18
Pityrosporum ovale	5	1	1	0	0	0	0	1	0	4	8
Scolescobasidium verruculosum	1	1	1	0	1	0	0	0	0	4	4

[a] Number of times each species was isolated during a particular sample period.

TABLE 3. *Recovery of fungal species at various sample periods*

Mission	Sample period	No. of fungal species recovered									Total no. of fungal species recovered			Average no. of fungal species recovered		
		A[a]			B			C								
		Y[b]	F	T	Y	F	T	Y	F	T	Y	F	T	Y	F	T
Apollo 14	F − 30[c]	6	15	21	5	11	16	5	8	13	10	31	41	5	12	17
	F − 15	3	11	14	2	8	10	2	11	13	5	25	30	2	10	12
	F − 0	2	6	8	1	6	7	3	9	11	4	16	20	2	9	9
	R + 0	1	1	2	0	0	0	2	2	4	2	3	5	1	1	2
	R + 16	1	10	11	0	10	11	1	9	10	1	22	23	1	10	11
Apollo 15	F − 30	3	10	13	3	16	19	3	10	13	6	25	31	3	12	15
	F − 5	5	7	12	4	6	10	2	5	7	9	12	21	4	6	10
	F − 0	4	5	9	5	6	11	5	2	7	9	10	19	5	4	9
	R + 0	1	1	2	1	1	2	2	1	3	3	3	6	1	1	2

[a] Astronaut A, B, or C.

[b] Y, Yeast and yeast-like; F, filamentous fungi; T, total fungi.

[c] F − 30, 30 days before flight; F − 15, 15 days before flight; F − 5, 5 days before flight; F − 0, the day of launch; R + 0, the day of recovery; R + 16, 16 days after recovery.

frequently isolated and requires study, even though it is a common air contaminant and therefore is probably not indigenous to man. All other recovered species are considered to be either transients or accidental contaminants.

It is of importance to note that four members of the genus *Candida* were the only yeast-like organisms frequently recovered. In addition to *C. albicans*, which is a well-recognized potential pathogen (29, 32), other species of *Candida* as well as *P. ovale* have variously been implicated with natural (13, 28, 31, 39) or experimental (6, 19, 22) infectious events. Analysis of the total microbial recovery during each sample period (Table 3) indicates that the relative importance of these species as possible sources of endogenous infections during space flight increased as the mission progressed.

A graphical summary of these data is presented in Fig. 1 and 2. The 41 different species recovered from the Apollo 14 crew 1 month prior to launch represents a more diverse microbial

TABLE 4. *Distribution of isolates of five genera of fungi recovered from Apollo 14 astronauts*[a]

Species	Sample period	Sample site											
		Scalp	Ear	Axilla	Hand	Navel	Groin	Toes	Nares	Throat	Gargle	Urine	Feces
Filamentous fungi													
Aureobasidium pullulans	F − 0												b[b]
Cladosporium cladosporioides	F − 30								B		B		
	F − 0				C					C			
	R + 16					C				C	B		
Cladosporium cucumerinum	F − 30								C				
	F − 0				B				C	B			
Cladosporium elatum	F − 30									A			
	F − 14												C
	F − 0									C			
	R + 16		C						B				A
Cladosporium sphaerospermum	F − 30								A	A			A
	F − 14									C			
Cladosporium colocasiae	F − 14			B		A							
Cladosporium herbarum	F − 14				C			C		C			BC
	F − 0							A					A
	R + 16					B				BC	B	A	B
Cladosporium macrocarpum	R + 16							AB		ABC	B		A
Scolescobasidium verruculosum	F − 30	B											
	F − 14						A						
	F − 0								C				
	R + 16	C											
Yeast and yeast-like													
Candida albicans	F − 30										C		C
	F − 14									C	AC		ABC
	F − 0									C	ABC		AC
	R + 0										A		AC
	R + 16									C	AC		C
Candida guilliermondii	F − 0									B			
Candida krusei	F − 14												C
Candida parapsilosis	F − 30				B								A
	F − 14		A										
Candida solani	F − 0		A	A				A					
Candida tropicalis	F − 0	B			AC	AB				AC	B		
Pityrosporum ovale	F − 30					BC	B	BC					
	F − 14							B					
	F − 0					C							

[a] Blank spaces indicate species not recovered.
[b] Astronaut A, B, or C.

load than was indicated by the 31 species recovered from the analagous Apollo 15 crew sample set. However, the average number of different species per individual was essentially the same for both crews. This indicates that the autoflora of the three Apollo 15 crewmembers were more alike than were the autoflora of the three Apollo 14 crewmembers.

Several authors (3, 8, 18, 30, 35, 40; P. E. Riely et al., Proc. Aerosp. Med. Ass., p. 275–276, 1967) have urged that special precautions must be taken to prevent the outbreak of diseases among crewmembers present in the sealed cabins of spacecraft for long periods of time. A preflight quarantine period has been suggested (21, 34, 37, 41) as one of the requirements for achieving this goal. The operational importance of such a quarantine period was demonstrated during the Apollo 13 mission when possible exposure to rubella disqualified one of the prime crewmembers 2 days before flight. As a result, the Apollo 14 and 15 crews were maintained in an environment designed to reduce the chances of exposure to infectious disease the last 21 days before launch. Two more sets of samples were collected during this restricted period as is shown in Fig. 1. The resulting data show that the mycological load was significantly reduced during this period and that a preflight low of 20 and 19 different species per Apollo 14 and 15 crew, respectively, was reached by the morning of launch.

Prior to entering the restricted preflight environment, the Apollo astronauts were required to conduct training missions at several remote sites, including tropical areas, volcanoes, and confined test chambers, which may have caused the 30-day preflight values to be unusually expanded. The preflight decrease in mycological load reflects a stabilization of the normal flora with a loss of transients and a reduction in the presence of unusual contaminants. Such a stabilizing reduction decreased the probability of future exogenous infections because the number of potential agents was greatly reduced. However, the probability of endogenous infection remained constant because there was no change in the incidence of the indigenous potential pathogen *C. albicans*. There were no incidences of mycological disease events either before or during either flight.

Similar analyses, such as the one conducted

TABLE 5. *Distribution of isolates of four genera of fungi recovered from Apollo 15 astronauts*[a]

Species	Sample period	Sample site											
		Scalp	Ear	Axilla	Hand	Navel	Groin	Toes	Nares	Throat	Gargle	Urine	Feces
Filamentous fungi													
Aureobasidium	F – 30									C[b]			
pullulans	F – 5							A					
	F – 0						A				BC		
Cladosporium	F – 30					B							
cladosporioides	F – 5							C					
Cladosporium	F – 30									B	B		
herbarum	F – 5	A						BC					
Yeast and yeast-like													
Candida	F – 30									A	AC		AC
albicans	F – 5					C				AC	AC		A
	F – 0									A	ABC		A
	R + 0									C	AC		AC
Candida krusei	F – 30												B
	F – 5										A		A
	F – 0												AB
Candida	F – 30	AC		A		C	A			C			B
parapsilosis	F – 5	A			A	C							A
	F – 0					C				C			B
Candida	F – 30					C							
tropicalis	F – 5									B	B		
	F – 0												B
	R + 0										B		
Candida scottii	F – 5											A	
	F – 0										C		
Pityrosporum	F – 0				A								
ovale													

[a] Blank spaces indicate species not recovered.
[b] Astronaut A, B, or C.

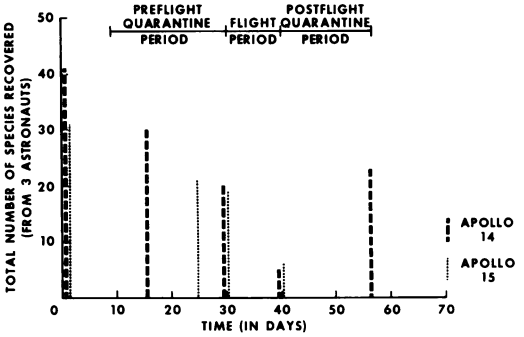

FIG. 1. *Total number of fungal species recovered from each set of Apollo 14 and 15 crew samples.*

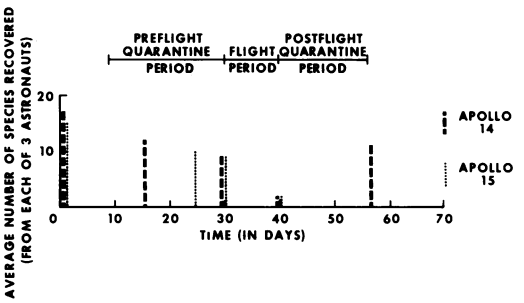

FIG. 2. *Average number of fungal species recovered from each set of Apollo 14 and 15 crew samples.*

for 38 days prior to entry of aquanauts into the Tektite I chamber, have shown that when no attempt is made to isolate crewmembers from re-contaminating sources, the fungal load remains essentially stable (9, 24). In studies where environmental restrictions have been imposed, increases in microbial bioburden have generally been demonstrated under conditions of poor hygiene (43), and in cases where adequate hygienic conditions have been maintained, a stabilization of the microbial population similar to that reported in this paper is often observed (2, 26, 34, 35, 36, 41). Unfortunately the environmental conditions for these studies vary so much from each other and from our work that the results are not comparable except in the broadest terms.

Analysis of the immediate postflight samples shows that the observable flora were reduced to less than one-third of the immediate preflight value (Fig. 1), indicating an equilibrium shift mediated by the space flight environment. During each flight the crew was in an environment of nearly pure oxygen at a pressure of one-third atmosphere. At the beginning of the flight the internal hardware components were quite clean (38), and the astronauts exercised a vigorous hygiene regimen throughout the mission. These factors could be expected to upset the established equilibrium whereby the astronauts lost microbial cells to, and picked up microbial cells from, the environment. The data show that the equilibrium was definitely shifted to favor a loss of fungi from the body which resulted in 90% of the immediate postflight isolates belonging to the two genera *Candida* and *Penicillium*. The above observations were based on data gathered from eight body surface swabs, a throat swab, a gargle sample, a fecal sample, and a urine sample. An analysis of which of these areas were most affected by the mission environment demonstrates that the loss was most prominent from the skin areas. The above observations tend to support the view, proposed by several authors (2–5, 8, 18, 20, 25, 26, 35), that microflora changes may occur in the spacecraft environment and that these changes may not be compatible with man's health and welfare on extended duration missions.

Few other confinement studies have been conducted in which the fungal load of healthy adults have been evaluated. Zaloguyev et al. (43) have reported changes in the incidence of *C. albicans* recovered from the skin and upper respiratory tract of subjects confined to a test chamber for 12 months. They report a 30-fold increase in the incidence of *C. albicans* in the pharynx the 1st month with a similar increase in the incidence of *C. tropicalis* starting the 6th month of confinement. They suggest that such confinement normalizes the bacterial flora and is invariably accompanied by the predominant growth of *Candida* fungi. They are referring here to an increase in the numbers of viable colony forming units recovered from a particular sample. We do not have comparable quantitative data, although we have found that the number of times this species is recovered, relative to all other fungi, is significantly elevated after space flight. For example, although all species of *Candida* comprise only 22% of all isolates recovered from the 30-day preflight samples, this same genus accounted for 56% of the immediate postflight isolates. Therefore, the 2-week space flights of Apollo 14 and 15 have resulted in the near disappearance of all species of fungi except *C. albicans*. If inflight antibiotic therapy was required under these conditions, the loss of competing bacterial species could result in an eventual overgrowth of *C. albicans* (21, 23, 27, 29, 33).

A set of samples was collected from the Apollo 14 astronauts 16 days after their return to earth. Analysis of these specimens (Table 1, Fig. 2) demonstrated that the number of different fungal species had returned to just slightly higher than the preflight low. During this 16-day postflight period the crew was maintained in total isolation within the lunar receiving

laboratory at the Johnson Space Center, but the astronauts were in close contact with 18 attendant personnel. The data indicate that the equilibrium had shifted so that the autoflora of the crew was numerically similar to that attained during the preflight confinement. There were no postflight clinical incidences and no evidence of microbial shock or gross contamination upon return to earth. It must be emphasized that 16-day postflight samples could not be taken after the Apollo 15 mission, for there was no longer a quarantine, and the crewmembers were not available for sampling. Therefore, the above data indicate only that microbial shock did not occur when astronauts were quarantined after space flight. It has not yet been shown that this would not occur in the absence of a postflight quarantine or after space flight of longer duration. This hypothesized reaction (3, 15, 16, 26, 34, 37, 41) of sparsely contaminated crewmembers to a sudden influx of a multitude of fungal species still remains a possibility to be evaluated during subsequent space flights.

The data presented in Tables 4 and 5 represent an enumeration of the species recovery incidence of the five frequently isolated genera. These data equate recovery with the sample collection period, the crewmember, and the area sampled. Analysis of the Apollo 14 data (Table 4) indicates that 16 different species of these five key genera were identified throughout the evaluation. Among the filamentous fungi, the various species of *Cladosporium* in general were quite evenly distributed throughout the sample sites, whereas *S. verruculosum* was recovered only from skin areas. Among the yeasts, *C. albicans*, *C. guilliermondii*, and *C. krusei* were recovered only from the oral cavity and the feces, whereas *P. ovale*, *C. parapsilosis*, *C. solani*, and *C. tropicalis* were more often recovered from skin swab samples. Except in the case of *C. albicans* in the feces of astronaut C, no species was recovered from a particular crewmember at every sample period.

Examination of the Apollo 15 recovery pattern demonstrates isolation of only nine species belonging to four of the frequently isolated genera. The filamentous fungi demonstrated a more expected pattern in that they were not recovered from the fecal samples. As in the Apollo 14 mission, *C. albicans* was the only species recovered from a crewmember at every sample period.

Most species are not consistently recovered from a particular site on a particular subject (Tables 4 and 5). For this reason we are unable to demonstrate intercrew transfer of fungal species either during isolation or during the space flight. We do not know what effect enclosure of astronauts in the space flight environment for long periods of time may have on the activity of fungal contaminants, although man-to-man transfer of pathogenic microorganisms were reported to have been regular occurrences in the closed ecological environment of the Apollo 7 thru Apollo 11 spacecraft (3, 18). No data were presented in these cited papers to support the above observation, and we have noted no intercrew transfer of potentially pathogenic fungi within the Apollo 14 and 15 crewmembers.

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